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BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Application Number: 09/645,706
Filing Date: August 24, 2000
Appellant(s): WOOD ET AL.

Janet Embretson
For Appellant

EXAMINER'S ANSWER

This is in response to the appeal brief filed 8/27/07 appealing from the Office action mailed 9/13/06.

(1) Real Party in Interest

A statement identifying by name the real party in interest is contained in the brief.

(2) Related Appeals and Interferences

The following are the related appeals, interferences, and judicial proceedings known to the examiner which may be related to, directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal:

Appeal of related child case 10/314,827 which is a continuation in part of the instant case.

(3) Status of Claims

The statement of the status of claims contained in the brief is substantially correct. However a few minor errors in appellants review of the prosecution are noted. These are: claims 19 and 40 were originally cancelled in the amendment of 8/11/03 not the amendment of 4/6/04, appellants review of the changes made in the amendment of 12/13/04 says that claims 1, 4-6, 9, 15, 18, 20-21, 24-37, 42-43, 45, 47, 60, 67, 69-71, 74, 76-78, and 80 were added but should indicate that these claims were amended and this list should additionally include claims 3, 11 and 12 also and appellants list of claims amended in the

response of 6/19/06 should include claim 93 also. Appellants list of the currently pending claims is correct.

(4) Status of Amendments After Final

The appellant's statement of the status of amendments after final rejection contained in the brief is correct.

(5) Summary of Claimed Subject Matter

The summary of claimed subject matter contained in the brief is correct.

(6) Grounds of Rejection to be Reviewed on Appeal

The appellant's statement of the grounds of rejection to be reviewed on appeal is substantially correct. However, the rejection of claims 47, 82, and 87 under 35 U.S.C. § 112, first paragraph is withdrawn herein as the amendments to claim 47 made in the after-final amendment of 2/12/07 limited these claims to a scope which had been previously deemed enabled, yet the Advisory Action inadvertently failed to indicate the withdrawal of this rejection for these claims. As such the enablement rejection to be reviewed is in fact: Whether claims 1, 3-6, 9, 11-12, 15, 20-21, 24-33, 35-39, 41-45, 60, 67, 69-70, 81, 86, 88, and 90-95 lack enablement under 35 U.S.C. § 112, first paragraph. Furthermore, appellants list whether claim 90, which depends on claim 1, is unpatentable under 35 U.S.C. § 112, second paragraph as a separate issue under 35 U.S.C. § 112,

second paragraph rejections to be reviewed; and whether claim 95, which depends on claim 67, is unpatentable under 35 U.S.C. § 103 (a) in view of Sherf et al., Zolotukhin et al., Donnelly et al., Pan et al., Cornelissen et al., and Hey et al. and whether claim 96, which depends on claim 74, is unpatentable under 35 U.S.C. § 103(a) over Sherf et al., Zolotukhin et al., Donnelly et al., Pan et al. Cornelissen et al., Hey et al., and further in view of Wood et al. as separate issues under 35 U.S.C. § 103 rejections to be reviewed yet the arguments section of appellants brief includes no subheading specifically identifying arguments for the separate patentability of these claims. As such a subheading and separate argument are required by 37 CFR 41.37 (c)(1)(vii), these are not considered to be separate issues from the rejections of the claims from which these claims depend.

GROUNDS OF REJECTION NOT ON REVIEW

The following grounds of rejection have not been withdrawn by the examiner, but they are not under review on appeal because they have not been presented for review in the appellant's brief.

Claims 91, 93 and 94 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-50 and 58-60 of copending

Application No. 10/314,827. See the Office Action of 12/19/05 for an explanation of the rejection.

(7) Claims Appendix

The copy of the appealed claims contained in the Appendix to the brief is correct.

(8) Evidence Relied Upon

5,670,356	Sherf et al.	9/1997
5,874,304	Zolotukhin et al.	2/1999
5,952,547	Cornelissen et al.	9/1999
6,169,232	Hey et al.	1/2001
WO 97/47358	Donnelly et al.	12/1997
WO 99/14336	Wood et al.	3/1999

Pan W. et al. "Vaccine Candidate MSP-I from Plasmodium Falciparum: a Redesigned 4917 bp Polynucleotide Enables Synthesis and Isolation of Full-Length Protein from *Escherichia coli* and Mammalian Cells", Nuc. Acids Res., Vol. 27, No. 4: 1094-1103 (1999).

Wood, K.V. et al., "Introduction to Beetle Luciferases and Their Applications", Journal of Bioluminescence and Chemiluminescence 4: 289-301 (1989).

WOOD, K.V., "The Chemical Mechanism and Evolutionary Development of Beetle Bioluminescence", Photochemistry and Photobiology, 62 (4): 662-673. (1995).

HASTINGS, J.W., "Biological Diversity, Chemical Mechanisms, and the Evolutionary Origins of Bioluminescent Systems", Journal of Molecular Evolution, 19 (3/4): 309-321 (1983).

(9) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

Claims 1, 3-6, 9, 11, 12, 15, 20, 21, 24-39, 41-45, 47, 60, 67, 69-71, 74, 76-78, 80-82, 85-88, and 90-96 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1 (from which claims 3-6, 9, 11, 12, 15, 20, 21, 24-39, 41-45, 60, 69, 70 81, 86, 89, and 90 depend), 47 (from which claims 71, 82, and 87 depend), 67 (from which claims 69, 70, 81, and 88 and 95 depend), 74 (from which claims 76, 77, 81,88 and 96 depend), and 78 (from which claims 80, 82, and 87 depend) are vague and indefinite in the recitation of "a reduced number of a combination of mammalian transcription factor binding sequences, intron splice sites, poly(A) addition sites and/or prokaryotic 5′ noncoding regulatory sequences" as without knowing all the possible sequences which are considered to be transcription factor binding sequences (TFBS), intron splice sites, poly(A) addition sites and prokaryotic 5′ noncoding regulatory sequences such a calculation is impossible as one could never obtain a count of the number of such sequence in any reference nucleic acid such that a skilled artisan could determine if the number in the first sequence is reduced relative to the number in the

second sequence. While there are clearly art defined specific sequences within each of these categories, each of them is an open-ended group of sequences which includes many unknown members and many such consensus sequences are defined differently by individual people such that the boundaries of variations which can occur within a sequence are often vague. For example, the sequence AATAAA is well known in the art to be the consensus poly(A) addition signal. However, many variants of this sequence also can act as poly(A) addition signals in some instances. Without knowing which of these are included in the scope of the term, a quantitative value for how many are present in any nucleic acid cannot be obtained and the value obtained for any specific nucleic acid by different individuals would be different if they used different definitions of what sequences are encompassed in each of these terms. Furthermore, while many transcription factors and their associated binding sequences are known in the art, new members are being added frequently such that the scope of the claims would change over time and what sequences constitute active variants of even the known sequences is vague and often context dependent i.e., dependent on the presence or absence of particular flanking sequences. The recitation of "wherein the mammalian transcription factor binding sequences are present in a database

of transcription factor binding sequences" to claims 1, 47, 67, 74 and 78 and the similar recitation in claim 90 does nothing to clarify what sequences are encompassed without recitation of the specific database and version. Different databases and even different versions of the same database have different sequences included such that the scope of the claims would vary depending on which database and version were used. Claims 91 and 92 (from which claims 93 and 94 depend) are similarly indefinite in the recitation of "known mammalian transcription factor binding sequences".

Claims 1, 3-6, 9, 11, 12, 15, 20-21, 24-33, 35-39, 41-45, 60, 67, 69, 70, 81, 86, 88, and 90-95 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for (1) a variant of a parent DNA molecule encoding a reporter polypeptide identical to a reporter polypeptide encoded by said parent DNA, having more than 25% of the codons altered and having a reduced number of transcription factor binding sequences, intron splice sites, poly(A) addition sites and prokaryotic 5' noncoding regulatory sequences than a mammalian codon optimized variant of the parent nucleic acid, (2) a variant of a parent DNA molecule encoding a luciferase having 90% identity to the polypeptide encoded by SEQ ID NO:2

and having more than 25% of the codons altered and having a reduced number of transcription factor binding sequences, intron splice sites, poly(A) addition sites and prokaryotic 5' noncoding regulatory sequences than a mammalian codon optimized variant of SEQ ID NO:2 or (3) to any nucleic acid which will hybridize to SEQ ID NO:9 under high stringency conditions and encode a polypeptide having luciferase activity, does not reasonably provide enablement for any variant DNA molecules encoding any reporter polypeptide having at least 90% identity to any wild type reporter polypeptide or any chloramphenicol acetyltransferase, *Renilla* luciferase, beetle luciferase, beta-lactamase or beta-galactosidase and having more than 25% of the codons altered and having a reduced number of transcription factor binding sequences, intron splice sites, poly(A) addition sites and prokaryotic 5' noncoding regulatory sequences than a mammalian codon optimized version of the parent nucleic acid. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims.

Claims 1, 3-6, 9, 15, 20-21, 24-33, 35-39, 41-45, 60, 81, 86, and 89-94 are so broad as to encompass any variant DNA molecules encoding any reporter polypeptide having at least 90% identity to a wild type reporter polypeptide (Claims 91, 93, and

4) or any chloramphenicol acetyltransferase, *Renilla* luciferase, beetle luciferase, beta-lactamase or beta-galactosidase (Claims 1, 3-6, 9, 15, 20-21, 24-33, 35-39, 41-45, 60, 81, 86, 89, 90 and 92) while claims 11, 12, 67, 69, 70, 88 and 95 are so broad as to encompass any variant DNA molecules encoding any luciferase polypeptide having at least 90% identity to any beetle luciferase. The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of variant nucleic acids broadly encompassed by the claims. Since the amino acid sequence of a protein determines its structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires a knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (i.e. expectedly intolerant to modification), and detailed knowledge of the ways in which the proteins' structure relates to its function. However, in this case the disclosure is limited to modifying the nucleic acid sequence of a desired gene without changing the encoded protein sequence or to making only a very limited number of modifications of the encoded amino acid sequence.

While recombinant and mutagenesis techniques are known, it is not routine in the art to screen for multiple substitutions or multiple modifications, as encompassed by the instant claims, and the positions within a protein's sequence where amino acid modifications can be made with a reasonable expectation of success in obtaining the desired activity/utility are limited in any protein and the result of such modifications is unpredictable. In addition, one skilled in the art would expect any tolerance to modification for a given protein to diminish with each further and additional modification, e.g. multiple substitutions.

The specification does not support the broad scope of the claims which encompass any variant DNA molecules encoding any reporter polypeptide having at least 90% identity to any wild type reporter polypeptide or any chloramphenicol acetyltransferase, *Renilla* luciferase, beetle luciferase, beta-lactamase or beta-galactosidase or any luciferase having at least 90% identity to any beetle luciferase because the specification does **not** establish: (A) regions of the protein structure which may be modified without effecting activity; (B) the general tolerance of any protein to modification and extent of such tolerance; (C) a rational and predictable scheme for modifying any residues with an expectation of obtaining the

desired biological function; and (D) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including any variant DNA molecules encoding any reporter polypeptide having at least 90% identity to any wild type reporter polypeptide or any chloramphenicol acetyltransferase, *Renilla* luciferase, beetle luciferase, beta-lactamase or beta-galactosidase or any luciferase having at least 90% identity to any beetle luciferase. The scope of the claims must bear a reasonable correlation with the scope of enablement (In re Fisher, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of variant nucleic acids having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See In re Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

Claims 1, 3-6, 9, 11, 12, 15, 20, 21, 24-39, 41-45, 60, 67, 69, 70, 81, 86 and 90-95 are rejected under 35 U.S.C. 103(a) as being unpatentable over Sherf et al. (US Patent 5,670,356) in view of Zolotukhin et al. (US Patent 5,874,304), Donnelly et al.

(WO 97/47358), Pan et al., Cornelissen et al. (US Patent 5,952,547), and Hey et al. (US Patent 6,169,232).

Sherf et al. teach a modified firefly luciferase gene in which 14% of the codons have been altered without altering the protein coding sequence such that the altered sequences were designed to optimize the codon selection for human host cells and eliminate restriction sites and sequences which encode transcription factor binding sites for known mammalian transcription factors including ATF, AP1, Sp1, AP2 etc. which would interfere with its genetically neutral behavior expected of a reporter gene. The altered gene includes at least 6 fewer transcription factor binding sites and was inserted into several mammalian expression vectors. The altered gene is transcribed and translated efficiently in mammalian host cells. The altered luciferase differs from the variant nucleic acids of the claims in that 25% or more of the codons were not altered. Sherf et al. further disclose that similar modifications could be made to other luciferase genes including click beetle luciferase genes.

Zolotukhin et al. teach a modified *Aequorea victoria* GFP gene in which 37% of the codons have been altered (and optionally up to even 80-90% may be altered) without altering the protein coding sequence such that the altered sequences were designed to optimize the codon selection for human host cells.

The optimized gene is inserted into an expression vector including a Kozak consensus sequence preceding the ATG initiation codon which optionally may include a multiple cloning site positioned between the promoter and the humanized GFP gene and/or downstream of the GFP gene. The altered gene preferably includes CTG codons encoding leucine, GTG or GTC codons encoding valine, GGC codons encoding glycine, ATC codons encoding isoleucine, CCT codons encoding proline, CGC codons encoding arginine, AGC codons encoding serine, ACC codons encoding threonine, and GGC or GGT codons encoding alanine and is transcribed and translated 5-10 times more efficiently in human cells than the wild type gene.

Donnelly et al. teach a modified hepatitis C virus core antigen gene in which 61% of the codons have been altered without altering the protein coding sequence such that the altered sequences were designed to optimize the codon selection for human host cells and eliminate sequences which encode undesired sequences (such as ATTTA sequences, intron splice sites, etc.) generated by the alteration of the natural codons (see pages 17-18).

Pan et al. teach a modified *Plasmodium falciparum* gene in which a large number of the codons have been altered without altering the protein coding sequence such that the altered

sequences were designed to optimize the codon selection for human host cells and eliminate sequences which might be detrimental to transcription and translation of the synthetic gene including sequences of promoters, poly A signals, intron splice sites and long runs of purines which might act as transcriptional termination sequences (see pages 1095). It should be noted that the elimination of undesired sequences was performed after the modification of the codon preference and thus would eliminate undesired sequences artificially introduced by the change in codons. The modified gene was successfully expressed in a variety of host cells (see page 1096) while expression of the unmodified gene has turned out to be difficult if not impossible (see page 1095).

Cornelissen et al. teach a modified *Bacillus thuringiensis* gene in which a small number of the codons have been altered without altering the protein coding sequence such that the altered sequences were designed to eliminate sequences which might be detrimental to transcription and translation of the synthetic gene and particularly to eliminate sequences of cryptic promoters or DNA regulatory elements thereof which specifically interact with nuclear proteins (i.e., transcription factor binding sequences), see column 5, line 55 - column 6,

line 15), and intron splice sites. The modified gene was successfully expressed in transgenic plants.

Hey et al. teach a plant sink protein gene in which a large number of the codons have been altered without altering the protein coding sequence such that the altered sequences were designed to optimize the codon selection for plant host cells and eliminate sequences which might be detrimental to transcription and translation of the synthetic gene including sequences of promoters, or elements thereof such as TATA box regions (i.e., a transcription factor binding sequence), poly A signals, intron splice sites, transcriptional termination sequences and runs of 4 or more pyrimidines which might interfere with transcription (see columns 9-12). It should be noted that the elimination of undesired sequences was performed after the modification of the codon preference and thus would eliminate undesired sequences artificially introduced by the change in codons.

Therefore, it would have been obvious to further modify the luciferase gene of Sherf et al. to both increase the codon preference for humans as each of Zolotukhin et al., Donnelly et al., Pan et al. and Hey et al. each teach modifying a large percentage of the codons of a gene to be expressed in a host of interest and to remove potential promoter sequences,

transcription binding factor sites, polyadenylation sites and splice sites as each of Sherf et al., Donnelly et al., Pan et al., Cornelissen et al. and Hey et al. each teach modifying at least several codons of a gene to be expressed in a desired host cell to match the codon preference of the host cell and/or to eliminate undesired sequences in order to increase its expression in the desired host cell and therefore increase its usefulness as a reporter gene in human and other desired host cells. One would have had a reasonable expectation of success in view of the results of the cited references which show that such alterations of other genes substantially improve the levels of expression in a desired host.

Claims 18, 47, 71, 74, 76-78, 80, 82-85, 87, 88 and 96 are rejected under 35 U.S.C. 103(a) as being unpatentable over Sherf et al. (US Patent 5,670,356) in view of Zolotukhin et al. (US Patent 5,874,304), Donnelly et al. (WO 97/47358), Pan et al., Cornelissen et al. (US Patent 5,952,547), and Hey et al. (US Patent 6,169,232) as applied to claims 1, 3-6, 9, 11, 12, 15, 20, 21, 24-39, 41-45, 60, 67, 69, 70, 81, 86 and 90-95 above, and further in of Wood et al. (WO 99/14336).

Sherf et al., Zolotukhin et al., Donnelly et al., Pan et al., Cornelissen et al., and Hey et al. are discussed above. Sherf et al. teach that additional genes encoding luciferase can

be similarly optimized for human expression. Wood et al. teach a gene encoding a yellow green click beetle luciferase gene (wild-type Luc*Ppl*YG) having 100% identity to SEQ ID NO:23 and 97% identity to the protein encoded by SEQ ID NO:2 herein. Therefore, it would have been obvious to one of skill in the art to optimize the expression of the yellow-green click beetle luciferase gene of Wood et al. in human cells as taught by the combined disclosures of Sherf et al., Zolotukhin et al., Donnelly et al., Pan et al., Cornelissen et al., and Hey et al.

(10) Response to Argument

A.) Rejection of claims 1, 3-6, 9, 11, 12, 15, 20, 21, 24-39, 41-45, 47, 60, 67, 69-71, 74, 76-78, 80-82, 85-88, and 90-96 under 35 U.S.C. 112, second paragraph.

Appellant's argue that those skilled in the art, even in the absence of Appellant's specification, understand the metes and bounds of the phrases: transcription factor binding sequences (TFBS), intron splice sites, poly(A) addition sites, and prokaryotic 5′ noncoding regulatory sequences, as they are conventionally used in the art and argue that these terms are in fact used in the references cited by the examiner in the 103 rejection. It is acknowledged that these terms are widely used within the art including within the references cited in the 103 rejections. However, in the art these terms define a group of

sequences related by function. The art does not define clearly **what** sequences are included in each group. Since appellants invention requires a skilled artisan to **quantify** the number of such sequences it is imperative that the artisan know explicitly what sequences are to be included and what sequences are not so one can in fact count them. While the art clearly defines **some** specific sequences which fall into each group (for example AAUAAA as a polyadenylation sequence) many other sequences may have the same function and not all such sequences are known and taught by the art. Furthermore, the ability of many such sequences to function is often dependent upon the context in which they are found (i.e., surrounding sequences) such that the same sequence may be functional in one context but not in others. It should be noted that changing the group of sequences defined within each of these terms (whether the result of addition of a newly discovered sequence(s) or simply the selection of a different list which either more narrowly or more expansively defines known consensus sequences) will alter the scope of nucleic acids encompassed by the instant claims. Addition of new members to any of these groups (or other alterations of the list of sequences) can increase (or decrease) the number of total sequences in either one or both of the synthetic nucleic acid or the parent nucleic acid. Note if the

new member were present one or more times only in the parent nucleic acid, a sequence that did not meet the limitations of the claims if searched without the new member might in fact meet the limitations of the claims when the new member was added and conversely if the new member were present one or more times only in the synthetic nucleic acid, a sequence that did meet the limitations of the claims if searched without the new member might in fact not meet the limitations of the claims when the new member was added. As such the SAME synthetic nucleic acid could be considered encompassed if one set of sequences were used but not encompassed if the set of sequences were altered and it is not clear what set of sequences appellants claims are intended to encompass.

Appellants further argue that there is nothing intrinsically wrong in using functional language, defining something by what it does rather than by what it is, in drafting patent claims and courts have recognized the practical necessity for the use of functional language. This is not persuasive because the examiner never stated that the use of functional language is always indefinite but that in the instant situation that this language is indefinite as the claims recite **a reduced number** of these sites requiring that a skilled artisan be capable of counting them. This is only possible if the sites

can be identified by some means besides experimental testing for the function as the number of subsequences of any reporter gene is so enormous that this could not be achieved. Furthermore, for the function of transcription factor binding, testing for function is further problematic because the specification does not define the scope of transcription factors to which binding is to be tested. The specification describes using a computer to scan the sequence of a gene for specific subsequences but does not identify a specific group to be scanned for.

Appellants argue that it is disclosed that transcription regulatory sequences (TRS) refers to nucleic acid sequences associated with the function of a transcription regulatory element, and that such sequences are typically recognizable. However, while transcription regulatory element are typically recognizable as sequence motifs, or corresponding to known consensus sequences, the problem in the instant situation is that the specification does not define what sequence motifs or consensus sequences should be used and if different sets are used the scope of the claims changes. Appellants used a specific set of such sequences in the Examples in the specification but the claims are not limited to the use of this set. While as appellants argue, TRS can be identified using databases and software, and pages 48-50 define a particular set

of sequences which can be used as splice sites, poly(A) sequences and prokaryotic 5'noncoding regulatory motifs, the claims do not define particular databases and software or particular sequences and the claims are not limited to those used in the specification. The Examiner has previously suggested amending the claims to define a particular set of sequences to overcome the instant rejection but appellants have declined to adopt the examiner's suggestion.

Appellants argue that although there may be new members added to the group of "mammalian transcription factors" over time, the independent claims in the present application provide that the synthetic nucleic acid molecules have a reduced number of a combination of mammalian transcription factor binding sequences, as a result of codon replacement of at least 25% of the codons of a parent reporter nucleic acid sequence with mammalian high usage codons and mammalian codons that are not high usage. Thus, Applicant's synthetic polynucleotides are readily recognized by one of skill in the art. However, this is not persuasive as not all nucleic acids having at least 25% of the codons of a parent reporter nucleic acid sequence with mammalian high usage codons and mammalian codons that are not high usage are encompassed by the claims. The claims also require that the claimed polynucleotide have a **reduced number** of

mammalian transcription factor binding sequences. It is this limitation that is indefinite as addition of new members to the group of mammalian transcription factor binding sequences as well as lack of clarity in what sequences are within known mammalian transcription factor binding sequences can either increase the number of total mammalian transcription factor binding sequences in one or both of the synthetic nucleic acid or the parent nucleic acid.

The Board is requested by appellants to consider that Example 1 of the specification discloses that synthetic click beetle luciferase sequences were prepared that had a reduced number of a combination of mammalian transcription factor binding sequences, intron splice sites, poly(A) addition sites and prokaryotic 5' noncoding regulatory sequences and as described in the declaration of Monika Wood, she determined the number of mammalian transcription factor binding sequences in the luc^+ sequence of Sherf et al. (U.S. Patent No. 5,670,356), using software and a database that are available to the public and comparable to those disclosed in the application. However, it has never been the examiner's contention that given a clear set of sequences to be searched that calculation of the number was not possible, but that the claims are indefinite absent a clear definition of what sequences are encompassed by these

terms. In both of the above examples, a specific list of sequences was used, but in the claims no such list is provided and thus the claims are indefinite as the scope will be different when different list of sequences are included.

B.) Rejection of Claims 1, 3-6, 9, 11, 12, 15, 20-21, 24-33, 35-39, 41-45, 60, 67, 69, 70, 81, 86, 88, and 90-95 under 35 U.S.C. 112, first paragraph, for lack of enablement of the full scope of the claimed invention.

Appellants argue that prior to the filing date of the present application, nucleotide sequences encoding amino acid sequences for various reporter polypeptides were known, the relative frequency of codons employed in different organisms was known, TFBS, intron splice sites, poly(A) addition sites and prokaryotic 5' noncoding regulatory sequences were known, and the specification teaches several representative species of luciferase mutants with amino acid substitutions relative to a wild type sequence, which representative proteins have reporter activity and that numerous substitutions have been introduced into beetle luciferases without affecting the reporter property of the substitution variants. For instance, in U.S. Patent No. 6,602,677, five mutant luciferases are disclosed that have 12, 21, 32, 37, and 37 substitutions, respectively, relative to a parent luciferase. Furthermore, several of the parent

luciferases of the specification i.e., GRver2, GRver5, GRver5.1, RDver2, RDver5, RDver5.1, RDver5.2, and RD156-1H9 all have from 1-9 substitutions relative to a parent luciferase. Likewise, numerous substitutions have been introduced into other reporter proteins, such as GFP.

First it should be noted that the enablement rejection does not suggest or argue that given a specific reporter gene that a skilled artisan could not make a synthetic nucleic acid as claimed that encodes the same amino acid sequence. While, a skilled artisan would not be able to make and use a variant of a parent DNA molecule encoding a reporter polypeptide identical to a reporter polypeptide encoded by said parent DNA, having more than 25% of the codons altered and having a reduced number of transcription factor binding sequences, intron splice sites, poly(A) addition sites and prokaryotic 5' noncoding regulatory sequences if the art worker could not recognize or understand what sequences are mammalian transcription factor binding sequences, intron splice sites, poly(A) addition sites and prokaryotic 5' noncoding regulatory sequences, the instant rejection of the claims was made to address a different problem as this problem is clearly addressed by the 112, 2nd rejection above (Note if the scope of sequences encompassed by these terms is deemed clear to a skilled artisan, there would be no problem

for a skilled artisan to construct a nucleic acid as claimed that encodes the same polypeptide as the parent nucleic acid). The instant rejection was presented to address the issue that the specification fails to enable a skilled artisan to construct polynucleotides encoding the full scope of variants of the polypeptide encoded by the parent nucleic acid recited in the claims. Furthermore, it should be noted that the instant rejection does not suggest that the specification does not enable polynucleotides encoding all variants of the polypeptide encoded by the parent nucleic acid recited but merely that it does not enable the full scope of variants of the polypeptide encoded by the parent nucleic acid recited in the claims.

Appellants arguments are noted however the scope of enablement is not commensurate in scope with the claims. The rejected claims are not limited to any particular chloramphenicol acetyltransferase, beetle luciferase, beta-lactamase or beta-galactosidase. The extent of art guidance with regard to regions of any particular reporter polypeptide which can be successfully modified while retaining reporter activity varies widely. While some reporters such as the North American firefly (i.e., *Photinus pyralis*) or click beetle (i.e., *Pyrophorus plagiophthalamus*) luciferases and GFP have been extensively modified and the art provides a substantial amount

of guidance for making active variants of these specific reporters; for many other reporter polypeptides, including many other luciferases (for example coelenterate luciferases) beta-lactamases and beta-galactosidases, the amount of guidance provided by the art is highly limited at best and for each of these groups of reporter proteins there is little indication that art recognized guidance for well known members of the group is sufficient to provide guidance to creating variants of all members of the class as these classes of reporters include proteins which differ substantially in structure from the known species. For evidence of the diversity of luciferases as a group and even the diversity of specifically beetle luciferases see particularly (Hastings, Wood (1995), and Wood et al., J. Biolum. Chemilum. 4:289, 1989, all of which were cited in applicants IDS of 7/10/06). Hastings teaches that bioluminescence has evolved many times and that there are:

> "many chemically different luciferins and luciferases, isolated from luminous organisms in different phylogenetic groups" (page 310),
>
> "The luciferases in the seven systems listed in Table 1 range in molecular weight from about 21,000 to 420,000, and also appear to differ with regard to details of their catalytic reaction mechanisms" (page 310),
>
> "One of the more striking revelations of the table is the large number of bioluminescent groups for which the chemical nature of the luciferin remains unknown" (page 312),

and

> "it can be asserted with some confidence that systems having such different substrates and reaction mechanisms will have very different enzymes, with no common sequences or other similarities, and thus will have originated independently." (page 317)

Wood et al. teach with regard to sequence comparisons of click beetle and firefly luciferases that:

> "Our inability to demonstrate cross-hybridization of their corresponding nucleic acid in Southern blots suggested a significant degree of evolutionary divergence had occurred between the firefly and click beetle luciferases" (page 298),
>
> "Alignment of the amino acid sequences, deduced from the cDNA sequences, reveals a 47% identity between the two luciferases" (page 298),
>
> "Throughout the alignment there are no regions of especially high sequence similarity. Thus there is no indication of which regions may have been conserved owing to catalytic or structural constraints on the enzymes" (page 298),

and

> "the hydropathy plots of firefly and click beetle luciferase shown some similarities, but overall appear to be quite different" (page 298)

and Wood (1995) teach:

> "the biochemical paradigms of bioluminescence are based primarily on a limited number of systems, mostly of luminous bacteria, fireflies, marine coelenterates and *Cypridina*", (page 662)
>
> "the bioluminescence chemistries of luminous beetles and marine arthropods reveal no biological commonalities. The enzymes do not have similar physical structures or

> properties and the substrates of the reaction are vastly different" (page 662),
>
> "little is known of the luminescent chemistries in these terrestrial arthropods. Those most closely related taxonomically to beetles (Coleoptera) are flies (Diptera), of which the cave glowworms of Australia and New Zealand are best know. Bioluminescence in Diptera is based on a soluble luciferin-luciferase system and is ATP-dependent but the luciferin is not cross-reactive with beetle luciferin" (page 662),
>
> "amino acid sequences of the firefly luciferases are 67-98% identical to one another. The luciferases of the *Luciola* genus share about 90% sequence identity. The most closely related are the luciferases of *H. parvula* and *L. mingrelica* with 98% sequence identity, suggesting that *H. parvula* may in fact be a member of the *Luciola* genus. The luciferases of *L. noctiluca* and *P. pyralis* are 84% identical but only about 67% identical with the luciferases of *Luciola*" (page 665),
>
> "Lampyridae and Phengodidae both belong to the superfamily Cantharoidea: the luciferases of these families are 50% identical on average. For Elateridae, in the superfamily Elateroidea, the elaterid luciferase is 50% identical to the phengodid luciferase and 48% identical to the lampyrid luciferases" (page 665)

and

> "The level of dissimilarity among the beetle luciferases is large given the relative youth of this enzyme group. Only 27% of the amino acid sequence is conserved among all the cloned beetle luciferases." (page 665).

All of these disclosures clearly highlight that the diversity of luciferases as a group is very high and even the beetle luciferases are very diverse such that art guidance with regard to making variants of one or more of these specific genes would not likely be useful for making variants of other members of

these groups. Note claims limited to variants of specific reporter genes which are well characterized in the art and for which the art does provide substantial guidance regarding which amino acids can be modified and which cannot i.e., claims 18, 47, 71, 74, 76-78, 80, 82-85, 87, and 96, are not rejected.

Appellants argue that the disclosure of WO 99/14336 shows that it is routine in the art to screen for multiple substitutions or multiple modifications. However, this is not persuasive because the numbers of modifications present in the proteins addressed in these disclosures is well within the scope of what the examiner deemed enabled (i.e., a variant of a parent DNA molecule encoding a reporter polypeptide identical to a reporter polypeptide encoded by said parent DNA, having more than 25% of the codons altered and having a reduced number of transcription factor binding sequences, intron splice sites, poly(A) addition sites and prokaryotic 5' noncoding regulatory sequences than a mammalian codon optimized variant of the parent nucleic acid, (2) a variant of a parent DNA molecule encoding a luciferase having 90% identity to the polypeptide encoded by SEQ ID NO:2 and having more than 25% of the codons altered and having a reduced number of transcription factor binding sequences, intron splice sites, poly(A) addition sites and prokaryotic 5' noncoding regulatory sequences than a mammalian

codon optimized variant of SEQ ID NO:2 or (3) to any nucleic acid which will hybridize to SEQ ID NO:9 under high stringency conditions and encode a polypeptide having luciferase activity) but the instant claims include much more. A skilled artisan would be well aware that as the number of modifications increases the number of possible sequences **increases** exponentially while the number of active sequences **decreases** exponentially. Thus both the amount of experimentation necessary make and test all possibilities as well as the level of predictability changes quickly. Appellants argue in response to the conclusion that it would require undue experimentation to prepare variant reporters and screen them for activity, that it is the fact that the outcome of such a screening program may be unpredictable which is precisely why a program is carried out and thus it is unreasonable to contend that a program to locate biomolecules with target biological or physical properties would not be carried out by the art because the results cannot be predicted in advance. However, it is noted that appellants are not claiming the screening methods they are claiming the results of the screening methods which their own argument admits are unpredictable. Furthermore, while programs for making and testing for biomolecules with target biological or physical properties are routinely done in the art, the scope of

modifications used is vastly smaller in scope than the instant claims would require as even the most advanced techniques available at the time of the invention (i.e., high throughput mutagenesis and screening techniques such as those used in WO 99/14336) would allow for finding a few active mutants within several hundred thousand inactive mutants as is the case for the claims deemed enabled (despite even this being an enormous quantity of experimentation that would take a very long time to accomplish) but finding a few mutants within several billion or more as in the claims rejected would not be possible. It should be noted that even for the click beetle luciferase, the most extensively modified variants in the art (i.e., U.S. Patent No. 6,602,677) still retain substantially more identity to the parent luciferase than the 90% identity recited in applicants claims. Appellants respond to the above statements by arguing that the claims at issue in *In re Wands* and *Hybritech* were not screening claims, but rather methods of using antibodies with particular properties. However, there was nothing in these cases which suggested that a large amount of the scope of these claims could not be isolated by the screening methods disclosed in the art. However, in this case the art available screening methods would be useful only for producing variants of the parent reporter nucleic acid which encode proteins much more

similar to the parent nucleic acid than the full scope of what is claimed, particularly for the many uncharacterized reporter nucleic acids encompassed by the instant claims. For all reasons above, the claims are deemed to require undue experimentation to make and use the full scope of the claimed invention.

C.) Rejection of claims 1, 3-6, 9, 11, 12, 15, 20, 21, 24-39, 41-45, 60, 67, 69, 70, 81, 86 and 90-95 under 35 U.S.C. 103(a) as being unpatentable over Sherf et al. (US Patent 5,670,356) in view of Zolotukhin et al. (US Patent 5,874,304), Donnelly et al. (WO 97/47358), Pan et al., Cornelissen et al. (US Patent 5,952,547), and Hey et al. (US Patent 6,169,232).

Appellants on pages 31-35 of the brief include a long discussion of each of the cited references individually, focusing extensively on the particular differences in each of these references from the claimed invention. However, as the instant rejection is a 103 rejection over the combination of cited references and is not a 102 rejection or a 103 over any one of these references alone, this discussion will not be further commented on as it is not relevant to the instant rejection. Appellants arguments as to the deficiencies of the combination of references as a whole are addressed below.

Appellants argue that Cornelissen et al. teach away from the claimed invention because Cornelissen et al. teach that very few modifications in the coding region are required to substantially alleviate expression problems and therefore Cornelissen et al. cannot be combined with the other references' teachings. However, this is not persuasive in view of the fact that Cornelissen et al. was added to the rejection to expressly address appellants argument (made in their response of 9/26/05) that none of the previously cited documents had recognized that codon replacement may create additional transcription factor binding sites. Cornelissen et al. explicitly state in column 3:

> "However, wholesale (i.e., non-selective) changes in codon usage can introduce cryptic regulatory signals in a gene, thereby causing problems in one or more of the six steps mentioned above [note: which include transcription and translation] for gene expression, and thus inhibiting or interfering with transcription and/or translation of the modified gene in plant cells".

This explicit disclosure by Cornelissen et al. shows that appellants argument was in fact not true and the art did recognize that codon replacement can create additional transcription factor binding sites and that such regulatory sequences are clearly within the category of unwanted sequences which may decrease gene expression. It is acknowledged that Cornelissen et al. address this identified problem in a different fashion than that recited in the instant claims.

However, Hey et al., Donnelly et al. and Pan et al. all show that the art recognized that codon modifications can introduce a variety of different sequences which are unwanted within the synthetic gene and that additional codon modifications can be used to decrease or eliminate these unwanted sequences. These references clearly show that the art also understood appellants solution (i.e., wholesale changes in codon usage followed by additional modification to the codon optimized sequence to eliminate the unwanted sequence(s) which were introduced during the optimization) to the problem. The mere teaching of a second solution to a problem does not teach away from the first solution.

Appellants argue that the combination of Sherf et al. with the other references is only through impermissible hindsight based upon Appellant's disclosure as the examiner has not provided a good reason why a skilled artisan with no knowledge of Appellant's invention, would choose to modify a beetle luciferase gene, rather than a fluorescent protein coding sequence, hepatitis C virus gene, merozoite surface protein-1 gene, BT gene or sink protein gene. This argument is not persuasive. The art provides motivation for the codon optimization of a large variety of different types of genes and the disclosure of Sherf et al. clearly shows that beetle

luciferase genes are within this large group. Merely because the art provides reasons for optimizing other genes as well (such as the GFP gene, hepatitis C virus gene, merozoite surface protein-1 gene, BT gene or sink protein gene of the other cited references) does not in any way negate the teaching of Sherf et al. that a skilled artisan would codon optimize a beetle luciferase gene. The remaining references are clearly combinable with Sherf et al. as they are all drawn to such codon optimization procedures, the problems inherent in such procedures and the solutions to these problems.

Appellants argue that while there is a general teaching in the combination of cited documents to alter codons and/or remove certain undesired sequences in a selected sequence, none of the cited documents teaches or suggests that codon alterations, optionally in conjunction with removal of other disclosed sequences may create TFBS and that the Examiner has acknowledged that none of the cited documents explicitly teaches that codon replacements may create unwanted TFBS. Appellants further argue that none of the cited documents discloses or suggests iterative removal of TFBS from a codon altered gene of any type. However, it should first be noted the the examiner's previous acknowledgement that none of the cited documents explicitly teaches that codon replacements may create unwanted TFBS is in

fact incorrect. Cornelissen et al. in the statement quoted above do in fact explicitly teach that codon replacements may create unwanted TFBS. The portion of the Office Action cited by Appellants was in fact an unfornunate repeat of a statement made in a previous Office Action before Cornelissen was added to the rejection which in view of the addition was no longer entirely correct. However, even if this were not explicitly taught in the prior art, Hey et al., Donnelly et al. and Pan et al. all show that the art recognized that codon modifications can **introduce** sequences which are unwanted within the synthetic gene, that additional codon modifications can decrease the introduction of those sequences and Sherf et al. clearly teach that the presence of transcription factor binding sequences within a reporter gene is an unwanted feature as it may interfere with the desired genetic neutrality of the reporter gene (see column 8) and Cornelissen et al. further teach that the presence of transcription factor binding sequences within a gene is an unwanted feature as it may interfere with transcription and or translation such that a skilled artisan would have understood that codon modification may introduce TFBS and that these could be removed by further codon modifications. Furthermore, it is obvious on its face that anytime a gene sequence is altered that one necessarily creates new sequences

which were not previously present and that merely by random chance some of these newly created sequences may be detrimental. It is even further obvious on its face that the more changes one makes, the higher the chances that such a detrimental sequence will be introduced. Sherf et al. made a smaller number of changes to codon selection and thus at least in his explicit teachings focused on the elimination of detrimental sequences present in the wild type sequence. However, the remaining art clearly would have motivated one of skill in the art to make more substantial changes in codon preference within the luciferase of Sherf et al. and the disclosures of Hey et al., Donnelly et al. and Pan et al. would have clearly led a skilled artisan who did so to scan not only the wild type sequence for the unwanted transcription factor binding sites but also the codon optimized version thereof. As such while the art does not explicitly teach iterative removal of TFBS from a codon altered gene, it does in fact clearly suggest it.

Appellants argue that the examiner is picking and choosing teaching from the cited documents that support the rejection while disregarding the differences. However, this is not in fact the case. The primary reference of the rejection (Sherf et al.) addresses the vast majority of the limitations of the rejected claims (i.e., optimization of a beetle luciferase

reporter gene to produce a synthetic gene encoding the same amino acid sequence as the wild type gene, introduction of specifically human preferred codons into the synthetic gene and removal of unwanted sequences including TFBS). The only differences between the instant claims and Sherf et al. are 1.) Sherf et al. did not modify at least 25% of the codons of the wild type gene and 2.) Sherf et al. did not teach iterative removal of the unwanted sequences (i.e., removal of unwanted sequences **introduced** by the initial optimization. The remaining references clearly provide both the motivation to alter the methods of optimizing the nucleic acid taught by Sherf et al. to include optimizing at least 25% of the codons of the wild type gene and to include iterative removal of the unwanted sequences as well as a reasonable expectation of success in doing so. The fact that the additional references used to show this happen to include references which teach optimization of other types of genes, or optimization for different hosts, and/or removal of different lists of unwanted sequences does not negate their relevance to the instant claims as they are all clearly addressing the problem of improved expression of genes in heterologous systems by alterations in the nucleotide sequence of the gene being expressed.

Appellants state that at best, the cited documents may suggest modifying a reporter gene over a large portion of the open reading frame with a view to generally remove undesired sequences introduced by codon replacement with preferred mammalian codons, and then with other mammalian codons. But the claims at issue are not directed to such a modified reporter gene. However, this is in fact exactly what appellants claims DO recite. It is not clear from this statement what appellants think the claims recite that is different. If the difference between this and appellants claims is the inclusion of specifically TFBS in the group of undesired sequences to be removed following codon optimization, the rejection and the references cited clearly teach the inclusion of TFBS undesired sequences which should not be present in a gene optimized for improved expression. Appellants state in the first paragraph of page 39 of the brief that none of the cited documents suggests that a polynucleotide that is modified by replacement of nonmammalian codons with mammalian codons be further modified by replacement with other, lower usage mammalian codons to reduce the number of introduced mammalian transcription factor binding sites. However, replacement with other, lower usage mammalian codons is not even a limitation of the claims. Furthermore Hey et al. clearly teach in column 10, line 63 - column 11, line 15

an optimization procedure that includes first optimizing all codons with the most preferred codon, then rescanning the optimized sequence for unwanted sites which may have been introduced and replacing the codons in these sites with the second or third choice (i.e., lower usage choice) for these positions. While Hey et al. is discussing optimizing a gene for expression in plants instead of for expression in mammals a skilled artisan would understand that the same basic strategy is used to optimize in either host with the only distinction being the use of a different preferred codon list.

Appellants further argue that the examiner has not provided a good reason why a skilled artisan would choose to modify the number of TRS, but not to reduce the number of glycosylation sites, restriction endonuclease sites, ATTTA sequences, long runs of purines, A and T sequences, TA and CG doublets, and blocks of G or C residues. Appellants argument is clearly off the point as the claims are not limited to eliminating ONLY transcription regulatory sequences or to eliminating specifically TFBS, intron splice sites, poly(A) addition sites and prokaryotic 5' noncoding regulatory sequences. They encompass all synthetic nucleic acids in which TRS sequences have been reduced relative to the wild type sequence and a hypothetical fully codon optimized version (i.e., the second

synthetic nucleic acid of the claims) regardless of whether other unwanted sequences have been reduced also. The examiner need not provide a reason not to reduce the number of other types of unwanted sequences as this is NOT a limitation of appellants claims. Appellants claims only require that the examiner provide a reason that a skilled artisan would choose to eliminate TRS which the rejection has clearly done. No selection of just TRS sequences from the list of unwanted sequences identified in the art is necessary. A skilled artisan could choose to reduce all of these unwanted sequences and the resulting nucleic acid would still be encompassed by appellants claims.

Appellants argue that none of the cited documents discloses or suggests the use of software to identify particular regulatory sites, such as mammalian transcription factor binding sequences, in a database of transcription factor binding sequences. However, this is not persuasive as most of applicants claims do not even mention the use of software to identify sites to be removed except for the inclusion of the language "wherein the mammalian transcription factor binding sequences are those present in a database of transcription factor binding sequence". However, this limitation does not require that these TFBS be identified by use of computer but

merely that the sites identified as TFBS (by whatever method the artisan chooses) are the same as those identified in one of more databases as being a TFBS. Furthermore, even for those claims that include the recitation of identification of regulatory sites using computer software (i.e., claims 90, 95, and 96), it is noted that the claims recite products not processes. Patentability of a product recited in product-by-process format is determined by the characteristics of the product itself not by the recited method. A nucleic acid in which the sites to be removed were identified by an undefined computer program would not differ in any respect from a nucleic acid in which the sites to be removed were identified by any other method.

Appellants argue that the cited art does not provide a reasonable expectation that codon optimization and the elimination of a variety of types of sequences which are detrimental to transcription and/or translation will improve the expression of a gene in a heterologous host as Sherf et al. found that the optimized gene did not uniformly increase luciferase activity in all mammalian cells. However, each of Zolotukhin et al., Sherf et al., Donnelly et al., Pan et al., and Hey et al. teach that codon optimization is done in the art to increase the expression of a desired gene in a heterologous host of interest. Thus the art clearly does expect that codon

optimization and the elimination of a variety of types of sequences which are detrimental to transcription and/or translation will improve the expression of a gene in a heterologous host. While it is acknowledged that one cannot be certain that the modifications will not have unexpected consequences (such as those of Pan et al. where Pan et al. had problems with cell death and secretion of the desired product), applicants are reminded that obviousness does not require an absolute certainty of success but only a reasonable expectation thereof. If the art found this procedure to be unsuccessful for its stated purpose it is unclear why so many have continued to use it. Note the examiner cited 5 references (Sherf et al., Zolotukhin et al., Donnelly et al., Pan et al., and Hey et al.) which teach optimizing substantial numbers of codons of a gene of interest and 5 references which teach elimination of sequences which might interfere with transcription and/or translation (Sherf et al., Donnelly et al., Pan et al., Cornelissen et al. and Hey et al.), and many others teaching this are present in the art not explicitly used in the rejection as well.

Appellants argue that the examiners previous statements that 1) it is obvious on its face that anytime a gene sequence is altered that one necessarily creates new sequences which were

not previously present and that merely by random chance some of these newly created sequences may be detrimental and it is even further obvious on its face that the more changes one makes, the higher the chances that such a detrimental sequence will be introduced and 2) the remaining art clearly would have motivated one of skill in the art to make more substantial changes in codon preference within the luciferase of Sherf et al. are contradictory and questions why would one make more changes when more changes would just increase the chances that a detrimental sequence would be introduced. However, this is not persuasive because the art clearly suggests that one will obtain greater increases in expression with higher levels of optimization. While this clearly increases the chances that a detrimental sequence will be **introduced**, the art also teaches how to remedy this potential drawback by rechecking the optimized sequence to eliminate newly created undesired sequences. Therefore, this potential drawback would not have prevented a skilled artisan from making more substantial changes in codon preference within the luciferase of Sherf et al.

Appellants argue that while it is relatively straightforward to remove ATTTA sequences, splice sites, restriction enzyme sites, prokaryotic promoter sequences, poly(A) signals, RNA polymerase termination signals, inverted

repeats, long runs of purines, TA and CG doublets, and blocks of G or C residues of more than about 4 residues, to remove a plurality of transcription factor binding sites, by replacing codons, the modifications must be selected in context, i.e., with reference to how those modifications impact adjacent sequences. However, this is not persuasive because while removing transcription factor binding sites might require more than a single nucleotide change to accomplish and might be more difficult than removing other sites, there is no reason to believe (and appellants do not present one) that a skilled artisan could not select alterations to the sequence which would eliminate these sites as well even if this required modifying more than one nucleotide of the sequence. Clearly Sherf et al. managed to eliminate several TFBS in the wild type luciferase gene successfully.

Appellants argue that the examiner has used the incorrect "obvious-to-try" standard as the references fail to provide any direction as to which of many possible choices of reducing the number of transcription regulatory sequences present in a parent nucleic acid sequence would be successful. First it is noted that in view of the Supreme Court decision in *KSR International Co., v. Teleflex, Inc.* an invention which is "obvious-to-try" may

in fact be obvious as well. Furthermore, appellants argument that the cited reference amount to a showing that the claims are at best "obvious-to-try" is not agreed with. The cited references clearly suggest that all unwanted sequences which are clearly disclosed as including transcription regulatory sequences be eliminated. While Cornelissen et al.'s disclosure teaches that only about 3% of the modifications are actually **required** to alleviate expression problems of most genes, in order to change only a limited number of codons one must actually **identify** which of the myriad possible sites are actually responsible for the problem (this is Cornelissen et al.'s solution to the problem). However this identification is not simple and routine so the majority of the art has used the other solution provided to the problem described in the instant rejection (i.e., using preferred codons at all (or most) codons and then eliminating unwanted sequences that such random optimization (i.e., without knowing which sites are responsible for the problem) introduced. Therefore the art clearly does guide the skilled artisan to optimize **all** codons of a gene and then eliminate **all** unwanted sequences that the optimization introduced.

Appellants argue that with respect to claims 1, 67, 91, and 92, that none of the cited references teaches or suggests that

the codons which differ in a first synthetic nucleic acid molecule relative to a second synthetic nucleic acid molecule are mammalian codons selected to result in the first synthetic nucleic acid molecule having a reduced number of a combination of different mammalian TFBS (claims 91 and 92), and optionally a reduced number of intron splice sites, poly(A) addition sites or prokaryotic 5' noncoding regulatory sequences (claims 1 and 67). However while the cited references may not use the same language, clearly all of Donnelly et al., Pan et al., and Hey et al. do in fact teach that the codons which differ in a first synthetic nucleic acid molecule relative to a second synthetic nucleic acid molecule are mammalian codons selected to result in the first synthetic nucleic acid molecule having a reduced number of a group of unwanted sequences which might potentially result in poor expression of the synthetic nucleic acid as each of these references clearly do teach creating a synthetic nucleic acid sequence and then further scanning it for the unwanted sequences and altering the codons a second time to eliminate these sequences. The disclosures of Sherf et al. and Cornelissen et al. clearly provide the necessary teachings to include TFBS within the group of unwanted sequences to be removed. As such the **combination** of references cited clearly do suggest that the codons which differ in a first synthetic

nucleic acid molecule relative to a second synthetic nucleic acid molecule are mammalian codons selected to result in the first synthetic nucleic acid molecule having a reduced number of a combination of different mammalian TFBS, and optionally a reduced number of intron splice sites, poly(A) addition sites or prokaryotic 5' noncoding regulatory sequences.

Finally with regard to claim 95 appellants argue that none of the cited references teach or suggest identifying in a wild type or second synthetic nucleic acid sequence intron splice sites selected from AGGTRAGT, AGGTRAG, GGTRAGT or YNCAGG, poly(A) addition sites having the sequence AATAAA, prokaryotic 5' noncoding regulatory sequences with the sequence TATAAT, or AGGA or GGAG if a methionine codon is within 12 bases 3' of the AGGA or GGAG, and mammalian transcription factor binding sequences that are in a database of transcription factor binding sequences, mutant transcription factor binding sequences and consensus transcription factor binding sequences, that are identified under parameters that allow for partial ambiguity with sequences in the database. However, appellants do not point out which part of this recitation they believe is missing from the cited prior art. First it should be noted that the inclusion of intron splice sites, poly(A) addition sites, and prokaryotic 5' noncoding regulatory sequences within the

sequences to be removed from the second synthetic nucleic acid of claim 95 is optional (see claim 67 from which claim 95 depends) and thus the recitation of the specific sequences of these elements is not in fact further limiting in this claim. Second with regard to the recitation "mammalian transcription factor binding sequences are in a database of transcription factor binding sequences, mutant transcription factor binding sequences and consensus transcription factor binding sequences, that are identified under parameters that allow for partial ambiguity with sequences in the database", Sherf et al. clearly teach eliminating transcription factor binding sites for known mammalian transcription factors including ATF, AP1, Sp1, AP2 all of which are well known TFBS that would be present in any database of TFBS and in fact Sherf et al. teach that the wild type luciferase gene was scanned using a database of consensus sequences for TFBS (see column 8, lines 65-67) which does allow for partial ambiguity with many sequences in the database (see Faisst and Meyer (1992) cited both by Sherf et al. and on applicants IDS of 6/13/01). Thus a skilled artisan would clearly have found it obvious to use this same database for removing TFBS from a synthetic nucleic acid created by substituting all or most codons of the gene of Sherf et al. with mammalian high usage codons.

D.) Rejection of claims 18, 47, 71, 74, 76-78, 80, 82-85, 87, 88 and 96 are rejected under 35 U.S.C. 103(a) as being unpatentable over Sherf et al. (US Patent 5,670,356) in view of Zolotukhin et al. (US Patent 5,874,304), Donnelly et al. (WO 97/47358), Pan et al., Cornelissen et al. (US Patent 5,952,547), and Hey et al. (US Patent 6,169,232) as applied to claims 1, 3-6, 9, 11, 12, 15, 20, 21, 24-39, 41-45, 60, 67, 69, 70, 81, 86 and 90-95 above, and further in of Wood et al. (WO 99/14336)

Appellants argue that Cornelissen et al. teach away from the claimed invention because Cornelissen et al. teach that very few modifications in the coding region are required to substantially alleviate expression problems and therefore Cornelissen et al. cannot be combined with the other references' teachings. However, this is not persuasive in view of the fact that Cornelissen et al. was added to the rejection to expressly address appellants argument (made in their response of 9/26/05) that none of the previously cited documents had recognized that codon replacement may create additional transcription factor binding sites. Cornelissen et al. explicitly state in column 3:

> "However, wholesale (i.e., non-selective) changes in codon usage can introduce cryptic regulatory signals in a gene, thereby causing problems in one or more of the six steps mentioned above [note: which include transcription and translation] for gene expression, and thus inhibiting or interfering with transcription and/or translation of the modified gene in plant cells".

This explicit disclosure by Cornelissen et al. shows that appellants argument was in fact not true and the art did recognize that codon replacement can create additional transcription factor binding sites and that such regulatory sequences are clearly within the category of unwanted sequences which may decrease gene expression. It is acknowledged that Cornelissen et al. address this identified problem in a different fashion than that recited in the instant claims. However, Hey et al., Donnelly et al. and Pan et al. all show that the art recognized that codon modifications can introduce a variety of different sequences which are unwanted within the synthetic gene and that additional codon modifications can be used to decrease or eliminate these unwanted sequences. These references clearly show that the art also understood appellants solution (i.e., wholesale changes in codon usage followed by additional modification to the codon optimized sequence to eliminate the unwanted sequence(s) which were introduced during the optimization) to the problem. The mere teaching of a second solution to a problem does not teach away from the first solution.

Appellants argue that the combination of Sherf et al. with the other references is only through impermissible hindsight

based upon Appellant's disclosure as the examiner has not provided a good reason why a skilled artisan with no knowledge of Appellant's invention, would choose to modify a beetle luciferase gene, rather than a fluorescent protein coding sequence, hepatitis C virus gene, merozoite surface protein-1 gene, BT gene or sink protein gene. This argument is not persuasive. The art provides motivation for the codon optimization of a large variety of different types of genes and the disclosure of Sherf et al. clearly shows that beetle luciferase genes are within this large group. Merely because the art provides reasons for optimizing other genes as well (such as the GFP gene, hepatitis C virus gene, merozoite surface protein-1 gene, BT gene or sink protein gene of the other cited references) does not in any way negate the teaching of Sherf et al. that a skilled artisan would codon optimize a beetle luciferase gene. The remaining references except Wood et al. are clearly combinable with Sherf et al. as they are all drawn to such codon optimization procedures, the problems inherent in such procedures and the solutions to these problems. Furthermore, Wood et al. is also combinable with Sherf et al. as Sherf et al. explicitly teach that additional genes encoding beetle luciferases can be similarly optimized for human expression (see particularly column 5, lines 44-56) and Wood et

al. clearly teach additional beetle luciferase genes (including a yellow-green click beetle luciferase gene encoding a protein 100% identical to SEQ ID NO:23 and 97% identical to the protein encoded by SEQ ID NO:2) which have particularly useful properties for use as a reporter. One of skill in the art attempting to apply the explicit teaching of Sherf et al. to optimize other beetle luciferase genes would clearly look to art disclosing beetle luciferase genes which have particularly useful properties for use as a reporter for selection of a gene to optimize.

Appellants argue that while there is a general teaching in the combination of cited documents to alter codons and/or remove certain undesired sequences in a selected sequence, none of the cited documents teaches or suggests that codon alterations, optionally in conjunction with removal of other disclosed sequences may create TFBS and that the Examiner has acknowledged that none of the cited documents explicitly teaches that codon replacements may create unwanted TFBS. Appellants further argue that none of the cited documents discloses or suggests iterative removal of TFBS from a codon altered gene of any type. However, it should first be noted the the examiner's previous acknowledgement that none of the cited documents explicitly teaches that codon replacements may create unwanted TFBS is in

fact incorrect. Cornelissen et al. in the statement quoted above do in fact explicitly teach that codon replacements may create unwanted TFBS. The portion of the Office Action cited by Appellants was in fact an unfornunate repeat of a statement made in a previous Office Action before Cornelissen was added to the rejection which in view of the addition was no longer entirely correct. However, even if this were not explicitly taught in the prior art, Hey et al., Donnelly et al. and Pan et al. all show that the art recognized that codon modifications can **introduce** sequences which are unwanted within the synthetic gene, that additional codon modifications can decrease the introduction of those sequences and Sherf et al. clearly teach that the presence of transcription factor binding sequences within a reporter gene is an unwanted feature as it may interfere with the desired genetic neutrality of the reporter gene (see column 8) and Cornelissen et al. further teach that the presence of transcription factor binding sequences within a gene is an unwanted feature as it may interfere with transcription and or translation such that a skilled artisan would have understood that codon modification may introduce TFBS and that these could be removed by further codon modifications. Furthermore, it is obvious on its face that anytime a gene sequence is altered that one necessarily creates new sequences

which were not previously present and that merely by random chance some of these newly created sequences may be detrimental. It is even further obvious on its face that the more changes one makes, the higher the chances that such a detrimental sequence will be introduced. Sherf et al. made a smaller number of changes to codon selection and thus at least in his explicit teachings focused on the elimination of detrimental sequences present in the wild type sequence. However, the remaining art clearly would have motivated one of skill in the art to make more substantial changes in codon preference within the luciferase of Sherf et al. and the disclosures of Hey et al., Donnelly et al. and Pan et al. would have clearly led a skilled artisan who did so to scan not only the wild type sequence for the unwanted transcription factor binding sites but also the codon optimized version thereof. As such while the art does not explicitly teach iterative removal of TFBS from a codon altered gene, it does in fact clearly suggest it.

Appellants argue that the examiner is picking and choosing teaching from the cited documents that support the rejection while disregarding the differences. However, this is not in fact the case. The primary reference of the rejection (Sherf et al.) addresses the vast majority of the limitations of the rejected claims (i.e., optimization of a beetle luciferase

reporter gene to produce a synthetic gene encoding the same amino acid sequence as the wild type gene, introduction of specifically human preferred codons into the synthetic gene and removal of unwanted sequences including TFBS). The only differences between the instant claims and Sherf et al. are 1.) Sherf et al. did not modify at least 25% of the codons of the wild type gene, 2.) Sherf et al. did not teach iterative removal of the unwanted sequences (i.e., removal of unwanted sequences **introduced** by the initial optimization and 3) Sherf's luciferase gene which was optimized is not a luciferase gene as recited in the instant claims. The remaining references clearly provide both the motivation to alter the methods of optimizing the nucleic acid taught by Sherf et al. to include optimizing at least 25% of the codons of the wild type gene and to include iterative removal of the unwanted sequences and to select a luciferase gene of Wood et al. to optimize as well as a reasonable expectation of success in doing so. The fact that the additional references used to show this happen to include references which teach optimization of other types of genes, or optimization for different hosts, and/or removal of different lists of unwanted sequences does not negate their relevance to the instant claims as they are all clearly addressing the problem of improved expression of genes in heterologous systems

by alterations in the nucleotide sequence of the gene being expressed.

Appellants state in the first full paragraph of page 45 of the brief that none of the cited documents suggests that a polynucleotide that is modified by replacement of nonmammalian codons with mammalian codons be further modified by replacement with other, lower usage mammalian codons to reduce the number of introduced mammalian transcription factor binding sites. However, replacement with other, lower usage mammalian codons is not even a limitation of the claims. Furthermore Hey et al. clearly teach in column 10, line 63 - column 11, line 15 an optimization procedure that includes first optimizing all codons with the most preferred codon, then rescanning the optimized sequence for unwanted sites which may have been introduced and replacing the codons in these sites with the second or third choice (i.e., lower usage choice) for these positions. While Hey et al. is discussing optimizing a gene for expression in plants instead of for expression in mammals a skilled artisan would understand that the same basic strategy is used to optimize in either host with the only distinction being the use of a different preferred codon list.

Appellants argue that the examiner has used the incorrect "obvious-to-try" standard as the references fail to provide any

direction as to which of many possible choices of reducing the number of transcription regulatory sequences present in a parent nucleic acid sequence would be successful. First it is noted that in view of the Supreme Court decision in *KSR International Co., v. Teleflex, Inc.* an invention which is "obvious-to-try" may in fact be obvious as well. Furthermore, appellants argument that the cited reference amount to a showing that the claims are at best "obvious-to-try" is not agreed with. The cited references clearly suggest that all unwanted sequences which are clearly disclosed as including transcription regulatory sequences be eliminated. While Cornelissen et al.'s disclosure teaches that only about 3% of the modifications are actually **required** to alleviate expression problems of most genes, in order to change only a limited number of codons one must actually **identify** which of the myriad possible sites are actually responsible for the problem (this is Cornelissen et al.'s solution to the problem). However this identification is not simple and routine so the majority of the art has used the other solution provided to the problem described in the instant rejection (i.e., using preferred codons at all (or most) codons and then eliminating unwanted sequences that such random optimization (i.e., without knowing which sites are responsible

for the problem) introduced. Therefore the art clearly does guide the skilled artisan to optimize **all** codons of a gene and then eliminate **all** unwanted sequences that the optimization introduced.

Appellants argue that none of the cited documents disclose or suggest a synthetic nucleic acid molecule which is capable of hybridizing to SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, or SEQ 1D NO:297 under high stringency conditions which encodes a luciferase (claim 18); a polynucleotide which hybridizes under medium stringency hybridization conditions to SEQ ID NO:9, SEQ ID NO:18, SEQ ID NO:297, SEQ ID NO:301 and comprises an open reading frame encoding a beetle luciferase polypeptide which has at least 90% amino acid sequence identity to a luciferase having SEQ ID NO:23 (claim 47); or a polynucleotide which hybridizes under medium stringency hybridization conditions to SEQ ID NO:9 or SEQ ID NO:297 and comprises an open reading frame encoding a luciferase polypeptide which has at least 90% amino acid sequence identity to a luciferase encoded by a parent nucleic acid sequence having SEQ ID NO:2 (claim 78). However, Wood et al. clearly disclose nucleic acids encoding wild-type Luc*Ppl*YG, a luciferase having 100% identity to SEQ ID NO:23 and 97% identity to the protein encoded by SEQ ID NO:2. Following the suggestions of Zolotukhin et al. with regard to specific codon

optimization choices for high level expression in human cells followed by modifications to eliminate undesirable sequences as taught by the secondary references, while not leading a skilled artisan to the specific nucleotide sequence of SEQ ID NO:9, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:18, SEQ ID NO:297, or SEQ ID NO:301 (as this would require that the art suggest all of applicants specific modification choices) would lead a skilled artisan to produce a optimized sequence which would hybridize to SEQ ID NO:9 under high stringency conditions as high stringency hybridization conditions still allow for a substantial number of positions (i.e., up to approximately 5% of the total; i.e., approximately 81 nucleotides in this case) in which the individual choices could be different.

Appellants argue that with respect to claim 74 that none of the cited references teaches or suggests that the codons which differ in a first synthetic nucleic acid molecule relative to a second synthetic nucleic acid molecule are mammalian codons selected to result in the first synthetic nucleic acid molecule having a reduced number of a combination of different mammalian TFBS, and optionally a reduced number of intron splice sites, poly(A) addition sites or prokaryotic 5' noncoding regulatory sequences. However while the cited references may not use the same language, clearly all of Donnelly et al., Pan et al., and

Hey et al. do in fact teach that the codons which differ in a first synthetic nucleic acid molecule relative to a second synthetic nucleic acid molecule are mammalian codons selected to result in the first synthetic nucleic acid molecule having a reduced number of a group of unwanted sequences which might potentially result in poor expression of the synthetic nucleic acid as each of these references clearly do teach creating a synthetic nucleic acid sequence and then further scanning it for the unwanted sequences and altering the codons a second time to eliminate these sequences. The disclosures of Sherf et al. and Cornelissen et al. clearly provide the necessary teachings to include TFBS within the group of unwanted sequences to be removed. As such the **combination** of references cited clearly do suggest that the codons which differ in a first synthetic nucleic acid molecule relative to a second synthetic nucleic acid molecule are mammalian codons selected to result in the first synthetic nucleic acid molecule having a reduced number of a combination of different mammalian TFBS, and optionally a reduced number of intron splice sites, poly(A) addition sites or prokaryotic 5' noncoding regulatory sequences.

With regard to claim 96 appellants argue that none of the cited references teach or suggest identifying in a wild type or second synthetic nucleic acid sequence intron splice sites

selected from AGGTRAGT, AGGTRAG, GGTRAGT or YNCAGG, poly(A) addition sites having the sequence AATAAA, prokaryotic 5' noncoding regulatory sequences with the sequence TATAAT, or AGGA or GGAG if a methionine codon is within 12 bases 3' of the AGGA or GGAG, and mammalian transcription factor binding sequences that are in a database of transcription factor binding sequences, mutant transcription factor binding sequences and consensus transcription factor binding sequences, that are identified under parameters that allow for partial ambiguity with sequences in the database. However, appellants do not point out which part of this recitation they believe is missing from the cited prior art. First it should be noted that the inclusion of intron splice sites, poly(A) addition sites, and prokaryotic 5' noncoding regulatory sequences within the sequences to be removed from the second synthetic nucleic acid of claim 96 is optional (see claim 74 from which claim 96 depends) and thus the recitation of the specific sequences of these elements is not in fact further limiting in this claim. Second with regard to the recitation "mammalian transcription factor binding sequences are in a database of transcription factor binding sequences, mutant transcription factor binding sequences and consensus transcription factor binding sequences, that are identified under parameters that allow for partial

ambiguity with sequences in the database", Sherf et al. clearly teach eliminating transcription factor binding sites for known mammalian transcription factors including ATF, AP1, Sp1, AP2 all of which are well know TFBS that would be present in any database of TFBS and in fact Sherf et al. teach that the wild type luciferase gene was scanned using a database of consensus sequences for TFBS (see column 8, lines 65-67) which does allow for partial ambiguity with many sequences in the database (see Faisst and Meyer (1992) cited both by Sherf et al. and on applicants IDS of 6/13/01). Thus a skilled artisan would clearly have found it obvious to use this same database for removing TFBS from a synthetic nucleic acid created by substituting all or most codons of the gene of Sherf et al. with mammalian high usage codons.

Finally appellants argue that none of the cited documents disclose or suggest a synthetic nucleic acid molecule which is capable of hybridizing to SEQ ID NO:9, SEQ ID NO:18, SEQ ID NO:297, or SEQ ID NO:301 under high stringency conditions and comprising an open reading frame encoding a luciferase polypeptide which has at least 90% amino acid sequence identity to a beetle luciferase having SEQ ID NO:23 (claim 83), or a polynucleotide which hybridizes under high stringency hybridization conditions to SEQ ID NO:9 or SEQ ID NO:297 and

comprises an open reading frame encoding a luciferase polypeptide which has at least 90% amino acid sequence identity to a polypeptide encoded by a parent nucleic acid sequence having SEQ ID NO:2 (claim 84). However, as was stated above with regard to claims 18, 47, and 78, Wood et al. clearly disclose nucleic acids encoding wild-type Luc*Ppl*YG, a luciferase having 100% identity to SEQ ID NO:23 and 97% identity to the protein encoded by SEQ ID NO:2. Following the suggestions of Zolotukhin et al. with regard to specific codon optimization choices for high level expression in human cells followed by modifications to eliminate undesirable sequences as taught by the secondary references, while not leading a skilled artisan to the specific nucleotide sequence of SEQ ID NO:9, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:18, SEQ ID NO:297, or SEQ ID NO:301 (as this would require that the art suggest all of applicants specific modification choices) would lead a skilled artisan to produce a optimized sequence which would hybridize to SEQ ID NO:9 under high stringency conditions as high stringency hybridization conditions still allow for a substantial number of positions (i.e., up to approximately 5% of the total; i.e., approximately 81 nucleotides in this case) in which the individual choices could be different.

(11) Related Proceeding(s) Appendix

No decision rendered by a court or the Board is identified by the examiner in the Related Appeals and Interferences section of this examiner's answer.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

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